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# The Role of Metals in Misfolding and Aggregation Processes: X-ray Spectroscopy and Numerical Simulations

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Recent X-ray Absorption Spectroscopy experiments designed to study the metal coordination mode in Cu-A $\beta$  and Zn-A $\beta$  complexes have been able to detect visible differences between Zn and Cu samples that are suggestive of dissimilar structural roles of the two ions. These findings indicate that metals can bind A $\beta$ -peptides in an intra- as well as in an inter-peptide coordination mode, hinting at a different aggregation propensity. First principle investigations of Cu-A $\beta$  and Zn-A $\beta$  complexes, based on *ab initio* molecular dynamics simulations of the Car-Parrinello type are set up to elucidate the molecular basis for this difference.

## 1 Introduction

Amyloidosis is a family of pathologies caused by the transition of certain endogenous proteins and peptides from their physiological soluble configuration to a pathological fibrillar state. The term describes a heterogeneous group of diseases (more than 20) characterized by extra-cellular deposition of fibril material<sup>1,2</sup>. Among them the Alzheimer disease (AD), a progressive and devastating neuro-degenerative pathology<sup>3</sup>, characterized by misfolding of well identified peptides called A $\beta$ -peptides. In AD, like in many other amyloidosis, an important, but not yet fully elucidated, role seems to be played by transition metals (mainly Cu<sup>+2</sup> and Zn<sup>+2</sup>) present in fairly large amount in neurological plaques. There exist in the literature conflicting statements about the ability of Cu<sup>+2</sup> and Zn<sup>+2</sup> in providing protection against or acting as promoters of plaques formation. The interest in elucidating the role of metals strongly increased after noticing that Cu<sup>+2</sup> and Zn<sup>+2</sup> chelators are capable of solubilizing A $\beta$ -aggregates. It is clear enough that the nature of the metal binding mechanism has significant consequences on the protein folding/unfolding and aggregation processes<sup>4</sup>. However, the experimental techniques used until very recently were not able to unambiguously reconstruct the local atomic structure around the metal.

The availability of third generation synchrotron radiation sources has significantly enlarged the range of application of X-ray Absorption Spectroscopy (XAS) in investigating structural properties of biological systems. XAS can be profitably used to study the environment of metal ions complexed with proteins and peptides in physiological conditions, owing to its chemical selectivity and sensitivity to the local atomic structure around the absorber. Furthermore an accurate analysis of the extended X-ray absorption fine structure (EXAFS) region of the spectrum in terms of single plus multiple scattering contributions has been proved to allow a clear-cut identification of the amino acid (a.a.) residues primarily bound to the metal<sup>5</sup>.

At the more fundamental level, describing the peculiar electronic properties of peptide-metal complexes in terms of quantum chemistry is necessary to really clarify the structural

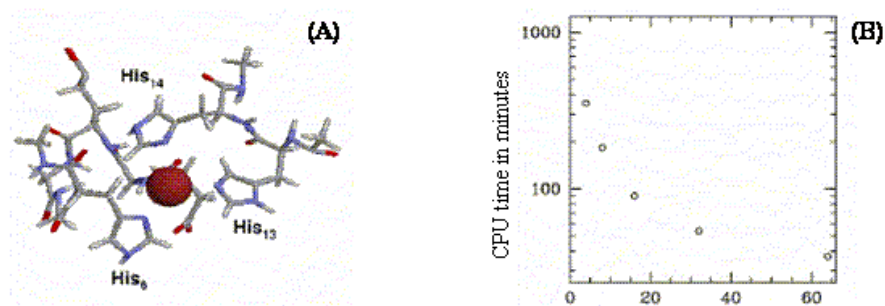


Figure 1. (A) The starting geometrical structure around Cu. (B) The scaling of the CPU running time of Espresso on the SGI Altix machine.

role of metal ions in misfolding and aggregation.

Density functional theory is the natural framework where a quantum mechanical description of the electronic structure of such compounds is possible. Special adjustments and refinements of the theory for application to bio-molecules are requested. The use of the plane-wave basis is particularly well suited for delocalized systems, like the organo-metallic molecules we are interested in, especially if coupled with the use of ultra-soft pseudo-potentials for the description of the electronic atom core. In this way the number of necessary plane waves can be kept at a tolerable level and Car-Parrinello (CP) *ab-initio* molecular dynamics (MD) can be efficiently carried out<sup>6,7</sup>. For reviews on the CPMD method see for instance<sup>8,9</sup>.

## 2 XAS experiments and simulations

I) Extensive XAS experiments were performed at the DESY-Hamburg EMBL facility on one of the two most frequently naturally produced  $\beta$ -amyloids, the  $A\beta_{1-40}$  peptide<sup>10</sup>. They show evidence that according to whether the peptide is complexed with  $Cu^{+2}$  or  $Zn^{+2}$  a different metal binding site structure is formed. In fact, while the geometry around Cu is stably consistent with an intra-peptide binding with three metal-coordinated Histidine residues, the Zn coordination mode depends on specific solution conditions. In particular, different sample preparations lead to different geometries around the absorber compatible with either an intra- or an inter-peptide coordination mode. This result reinforces the hypothesis that assigns different physiological roles to the two metals, with Zn favoring peptide aggregation and, hence, possibly plaque formation. Further XAS measurements<sup>11</sup> on selected, shorter portions of the whole  $A\beta_{1-40}$ -amyloid, in complex with  $Cu^{+2}$  and  $Zn^{+2}$ , have allowed to identify the specific a.a.'s primarily bound to the metal. Analysis of spectral data suggests the following structural geometries.

i)  $Cu^{+2}$  is penta-coordinated with 3 Histidines, and 2 oxygen atoms.

ii)  $Zn^{+2}$  is penta-coordinated with 4 Histidines and 1 oxygen atom.

Since only three Histidine residues are present in each  $A\beta$ -peptide the peculiar structure of the Zn environment indicates that Zn acts as a bridge between two peptides in an inter-molecular coordination arrangement.

II) *Ab initio* CPMD simulations represent an invaluable tool to validate the detailed structural model suggested by experiments. We then constructed an atomistic model system for the  $Cu^{+2}$ - $A\beta$ -peptide complex. In order not to have an impossibly large

system, of the first six a.a.'s the lateral chains of only the first (Aspartic acid), third (Glutamic acid) and sixth (Histidine) a.a., as well as of the 13<sup>th</sup> and 14<sup>th</sup> Histidine residue (His<sub>13</sub> and His<sub>14</sub>) are simulated in full atomic details. For the rest of the 1-6 a.a.'s only the backbone is retained. Furthermore none of the remaining a.a. is included. The C-terminal is capped with an NHCH<sub>3</sub> group, while the N-terminal of the 1-6 fragment is left open (with an ending NH group) to allow possible Cu binding with loss of the amide hydrogen. Structural information from XAS<sup>10,11</sup> and NMR<sup>12</sup> data were exploited in setting up the geometry around Cu which is schematically shown in Fig. 1 (panel A). The whole system, solvated with 125 water molecules, is contained in a box with a volume  $V=15 \times 20 \times 20 \text{ \AA}^3$ . In this way density is set equal to 1 g/cm<sup>3</sup>. All in all (i.e. including water) the simulated system comprises 494 atoms and 1369 electrons. A similar system with Zn replacing Cu is under test.

III) Simulations are performed using the CPMD Espresso code<sup>7</sup>. In the Table we show the performances of the code, all taken in the 16 nodes configuration, on two PC-clusters (Fermi1 in Rome and BEN in Trento) and the SGI Altix 4700 machine (in Munich). Fig. 1

machine	nodes	$t_{CPU}$ (s)
Fermi1	16	5880
BEN	16	4560
Altix	16	1080

(panel B) shows the scaling with the number of nodes of the CPU times on the Altix machine. The performances are for a not fully optimized code. The interesting outcome of this feasibility study is that with a moderately large number of nodes (say 64) on the SGI machine a fairly long CP trajectory (3-4 ps) of the large system described before can be collected in less that two months. Simulations of this length will allow to clarify the possible involvement of the N-terminal region of the A $\beta$ -peptide in binding the metal, as suggested by XAS data<sup>10,11</sup>.

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